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Soybean oil and crude protein levels for growing pigs kept under heat stress conditions

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ABSTRACT

This study was conducted to evaluate the effect of different levels of soybean oil (SO) and crude protein (CP) on performance and physiological and hormonal parameters of pigs in high-temperature environment. Eighty-four growing pigs with initial weight of 36.9 ± 3.0 kg were assigned to treatments in 2×3 factorial arrangement of dietary treatments plus 1 [two levels of CP, 18% and 15.5%, and three levels of SO, 1.5%, 3.0%, and 4.5% with all pigs kept at room temperature of 32 °C, and an additional treatment (control) with 18% CP and 1.5% SO with pigs kept at 22 °C], in a randomized block design with the block being the initial weight. In animals maintained in the high-temperature environment, there was no interaction between CP and SO levels in any of the variables evaluated. The addition of SO improved ($P < 0.05$) the average daily gain (ADG) and feed:gain (F:G), but did not affect the average daily feed intake (ADFI). The reduction of CP resulted ($P < 0.05$) in lower ADFI. Compared to animals kept at the comfort temperature, a high-temperature environment decreased the F:G ($P < 0.01$) of animals fed diets with 18% CP and 1.5% SO. However, increasing the SO level resulted in similar values of F:G. For animals kept in a high-temperature environment, CP can be reduced in diets supplemented with amino acids only in diets with high SO levels. The high-temperature affected ($P < 0.01$) body temperature and respiratory rate, but was not sufficient to change the levels of T3 and T4. It is concluded that SO levels should be increased in diets for animals kept in high-temperature environment, and the CP levels can be reduced only under these conditions, since supplemented with amino acids.

1. Introduction

For many years, environment, as well as management, health, genetics, and nutrition, has been considered to be a limiting factor for maximum animal production. Among domestic animals, pigs are more sensitive to high temperatures. It was known that this sensitivity is due to a combination of factors, among which are a poor thermoregulatory system, keratinized sweat glands, presence of a subcutaneous fat layer and intense metabolism. Therefore, maximum efficiency of pig production is compromised by conditions of high temperature, especially in heavier animals (Le Bellego et al., 2002; Quiniou et al., 2000).

Physiologically, pigs produce heat as a result of their production and maintenance functions. However, their body development and production efficiency are possible only if this body heat production is minimal (Tavares et al., 2000). To adapt to these conditions, the animals increase their respiratory frequency (Manno et al., 2006)

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or direct blood flow to the body periphery, raising the skin temperature (Carvalho et al., 2004). Another mechanism is to reduce food intake (Witte et al., 2000). This reduction is employed in an attempt to decrease production of body heat from metabolism. In practice, this reduction in food intake is related to lower performance of animals and a longer period of time taken to reach slaughter weight (Kiefer et al., 2005; Witte et al., 2000).

In this context, control of the thermal environment in the context of husbandry seems to be the most viable alternative for obtaining satisfactory results for pig production in hot regions. However, new nutrition practices have also been studied as an alternative in reducing the negative effects of heat stress.

Based on current knowledge, the use of modified diets is one of the nutritional strategies aimed at improving the productivity of pigs in hot periods. A reduction in crude protein with supplemental amino acids has been used to maintain performance and to reduce nitrogen excretion (Ferreira et al., 2007; Le Bellego et al., 2002; Zangeronimo et al., 2007). However, these diets can also be used to solve problems arising from a hot environment. This assumption is justified because the digestion of nutrients is a factor that generates heat, especially digestion of protein in comparison to carbohydrates and lipids (Stahly and Cromwell, 1986).

Besides the reduction of crude protein in diet, the inclusion of oil can also be an advantage, because the caloric increment is even lower (Li and Sauer, 1994; Noblet et al., 2001; Spencer et al., 2005). In this sense, a combination of these factors may be beneficial to animals kept in hot environments (Le Bellego et al., 2002). However, there has been little study on the effects of lipids in reduced crude protein diets containing synthetic amino acids. It is known that lipids have an influence on protein digestibility and amino acid availability (Almeida et al., 2007).
In this instance, an imbalance of amino acids could be involved and, consequently, loss in animal performance.

The aim was, therefore, to verify if diets with different levels of soybean oil, formulated with reduced crude protein supplemented with amino acids, can influence the performance and physiological parameters in growing pigs housed in a hot environment.

2. Materials and methods

The experiment was conducted at the Swine Experimental Center, Department of Animal Science, Federal University of Lavras, Lavras, Minas Gerais, Brazil. The experimental protocol was approved by the Bioethics Committee of the Federal University of Lavras.

2.1. Experimental design, animals, housing, and diets

Eighty-four barrows from a commercial line (Tempo × Topigs 40, TOPIGS, Helvoirt, The Netherlands) with initial weight of 36.9 ± 3.0 kg were used. The animals were housed in groups of two in two air-conditioned rooms in pens with concrete flooring, 1.38×2.82 m^2 in size, equipped with semi-automatic feeders and adjustable nipple drinkers. The experimental period lasted 30 d.

The rooms were equipped with automatic equipment (MT-530 Super, Full Gage Controls, UL Inc., US) responsible for the circulation of heated or cooled air in the room and infrared lamps and fans. All equipment was connected to a central panel, allowing the automatic adjustment of the internal temperature of the rooms. In the high temperature environment, the control panel was set to 32 °C, while in a thermoneutral environment, it was regulated at 22 °C. The relative humidity was set to be between 60% and 70% in both environments.

The air change of the rooms was constant, regulated by blowers and fans attached to a pipe with small holes for air distribution. The equipment automatically stayed on for 15 min and off for 2 min.

The temperature and relative humidity were monitored daily inside the rooms at 08:00, 13:00 and 18:00, by a maximum and minimum thermometer, barometer and dry and wet bulb and black globe thermometers (INCOTERM Thermo-hygrometer dry and wet bulb, code 5203.03.0.00; Porto Alegre, Brazil) placed at the center of the room and at a half height of the animals. The recorded values were then used to calculate the black globe humidity index (BGHI), according to Buffington et al. (1981), characterising the thermal environment in which the animals were kept.

The animals were assigned to treatments in 2×3 factorial arrangement of dietary treatments plus 1 [two levels of CP, 18% and 15.5%, and three levels of SO, 1.5%, 3.0%, and 4.5% with all pigs kept at room temperature of 32 °C, and an additional treatment (control) with 18% CP and 1.5% SO with pigs kept at 22 °C], in a randomized block design with six replicates of two animals per experimental pen. The criterion for the block was the initial weight of animals.

The experimental diets (Table 1) were corn and soybean meal based, containing vitamins, minerals and amino acids to meet the minimum requirements suggested for the lineage (Tempo × Topigs 40; TOPIGS, Helvoirt, The Netherlands). Water and feed were supplied ad libitum. Diet supply and orts were measured daily.

2.2. Sampling and measurement

Surface temperatures (neck, shoulder and leg) were measured in all animals, every 7 d, by an infrared thermometer (INCOTERM Digital Infrared Laser Thermometer Pointer—ST600, Porto Alegre, Brazil) orientated to the skin. The rectal temperature was measured by introducing a thermometer into the rectum of the animal for 1 min. The respiratory rate (breaths/minute) was obtained with the animal lying down by counting the flank movements of the animals for 15 s.

The animals were weighed at the beginning and end of the experiment. feed:gain (F:G) was obtained using the relationship between average daily feed intake (ADFI) and average daily gain (ADG) in the period.

At the end of the experiment, the animals' blood collection was performed by puncturing the orbital sinus with hypodermic needles $16G \times 1.27$ cm, without fasting,

Table 1
Composition of the experimental diets (as-fed basis).

Item	18.0% CP			15.5% CP		
	Soybean oil (%)			Soybean oil (%)		
	1.5	3.0	4.5	1.5	3.0	4.5
Ingredient (%)						
Corn	57.00	57.00	57.00	60.50	60.50	60.50
Soybean meal (45% CP)	30.70	30.70	30.70	24.40	24.40	24.40
Starch	7.50	4.00	0.50	10.20	6.70	3.20
Soybean oil	1.50	3.00	4.50	1.50	3.00	4.50
Dicalcium phosphate	1.35	1.35	1.35	1.35	1.35	1.35
Limestone	0.75	0.75	0.75	0.75	0.75	0.75
Salt	0.43	0.43	0.43	0.27	0.27	0.27
Trace mineral premix[a]	0.05	0.05	0.05	0.05	0.05	0.05
Vitamin premix[b]	0.10	0.10	0.10	0.10	0.10	0.10
DL-Met, 99%	0.06	0.06	0.06	0.09	0.09	0.09
L-Lys HCl, 78%	0.01	0.01	0.01	0.21	0.21	0.21
L-Thr,98%	0.08	0.08	0.08	0.17	0.17	0.17
L-Trp, 99%	0.00	0.00	0.00	0.04	0.04	0.04
Aurion[c]	0.30	0.30	0.30	0.30	0.30	0.30
Tylan[d]	0.03	0.03	0.03	0.03	0.03	0.03
Kaolin	0.13	2.13	4.12	0.00	1.80	3.80
Sodium bicarbonate	0.00	0.00	0.00	0.24	0.24	0.24
Antioxidant (BHT)	0.01	0.01	0.01	0.01	0.01	0.01
Composition						
GE (kcal/kg)[e]	3932	3935	3940	3913	3918	3921
ME (kcal/kg)[f]	3261	3262	3263	3274	3275	3276
CP (%)[e]	18.44	18.35	18.37	15.67	15.80	15.75
Ether extract (%)[e]	4.13	5.66	7.17	4.15	5.69	7.30
Ca (%)[f]	0.70	0.70	0.70	0.69	0.69	0.69
Available P (%)[f]	0.35	0.35	0.35	0.34	0.34	0.34
Na (%)[f]	0.19	0.19	0.19	0.19	0.19	0.19
Digestible Lys (%)[f]	0.90	0.90	0.90	0.90	0.90	0.90
Digestible Met (%)[f]	0.33	0.33	0.33	0.33	0.33	0.33
Digestible Thr (%)[f]	0.71	0.71	0.71	0.71	0.71	0.71
Digestible Trp (%)[f]	0.21	0.21	0.21	0.21	0.21	0.21
Digestible Arg (%)[f]	1.19	1.19	1.19	1.00	1.00	1.00
Digestible Val (%)[f]	0.79	0.79	0.79	0.68	0.68	0.68
Digestible Ile (%)[f]	0.74	0.74	0.74	0.63	0.63	0.63
Electrolyte balance (mEq/g)[g]	182	182	182	182	182	182

[a] Contributed per kilogram of diet: 0.25 mg of Se, 35 mg of Fe, 10 mg of Cu, 20 mg of Mn, 0.04 mg of Zn and 0.4 mg of I.
[b] Contributed per kilogram of diet: 8000 IU of vitamin A, 1200 IU of vitamin D_3, 20 mg of vitamin E, 2.5 mg of vitamin K_3, 1.0 mg of vitamin B_1, 4.0 mg of riboflavin (B_2), 2.0 mg of pyridoxine (B_6), 20 mcg of vitamin B_{12}, 25 mg of niacin, 10 mg of pantothenic acid, 0.6 mg of folic acid, 0.5 mg of biotin, 50 mg of vitamin C, and 0.125 mg of antioxidant.
[c] Contributed per kilogram of diet: 0.3 g of chlortetracycline hydrochloride, 0.225 g of sulfamethazine, 0.045 g of trimethoprim, and 0.3 g of citric acid.
[d] Contributed with 7.5 g of tyrosine/kg diet.
[e] Analyzed at in the Laboratory of Animal Nutrition, University Federal of Lavras (Minas Gerais, Brazil).
[f] Calculated according to Rostagno et al. (2005).
[g] Calculated according to Mongin (1980).

for determination of thyroid hormones (thyroxine and triiodothyronine—T3 and T4). Analyses of serum-free T3 and T4 were performed in a competitive chemiluminescence immunoassay (Immulite 2000® System; Siemens Healthcare Diagnostics Products, Gwynedd, UK).

2.3. Statistical analyses

The data were subjected to analysis of variance using the PROC GLM of SAS (v. 6.12; SAS Inst. Inc., Cary NC). The following models were used: $Y_{ijk}=\mu+B_i+P_j+O_k+PO_{jk}+e_{ijk}$ and $Y_{ij}=\mu+B_i+A_j+e_{ij}$, where Y is the dependent variable, μ is the mean, B is the block, P is the protein, O is the oil, PO is the interaction between protein and oil, A is the additional treatment and e is the residual error. Dunnett's test was performed to compare the control to each of the other treatments. Then, the Tukey test was used to test treatments in a factorial arrangements. The level of significant difference was set at $P<0.05$.

3. Results

The average temperature recorded during the experimental period was 32.3 ± 0.7 °C in the high temperature and 24.3 ± 0.9 °C in the thermoneutral environment. The relative humidity of the air was $60.8\pm3.6\%$ and $72.3\pm4.4\%$, respectively. The black globe humidity index

Table 2
Performance, corporal temperature, respiratory rate and serum-free T3 and T4 of growing pigs kept in high-heat environment fed diets with different levels of soybean oil (SO) and CP[1].

Item	Control	CP (%)		SO (%)			CV (%)	P-value		
		18.0	15.5	1.5	3.0	4.5		CP	SO	CP × SO
Average daily gain (g/d)	1017	936	954	911[b]	940[ab]	985[a]	5.51	0.42	0.01	0.18
Average daily feed intake (g/d)	2310	2311	2421	2372	2348	2378	6.63	0.04	0.91	0.21
Feed:gain	2.29	2.48	2.55	2.62[a*]	2.50[ab]	2.42[b]	7.05	0.30	0.04	0.59
Leg temperature (°C)	34.0	36.3*	36.0*	36.1*	36.2*	36.1*	1.42	0.22	0.80	0.78
Nape temperature (°C)	34.2	36.3*	36.2*	36.2*	36.4*	36.1*	1.45	0.48	0.44	0.22
Shoulder temperature (°C)	34.0	36.7*	36.6*	36.6*	36.7*	36.6*	1.14	0.42	0.73	0.26
Rectum temperature (°C)	39.3	39.5*	39.6*	39.5*	39.6*	39.5*	0.27	0.23	0.26	0.25
Respiratory rate (movements/min)	42.9	80.1*	80.8*	78.1*	85.2*	78.0*	9.92	0.84	0.16	0.23
T3 (pg/mL)	1.38	1.34	1.41	1.37	1.40	1.35	10.22	0.36	0.85	0.34
T4 (ng/dL)	1.50	1.51	1.54	1.54	1.55	1.49	15.19	0.58	0.57	0.96

* Differ from the control ($P < 0.05$).
[1] Control = animals kept at a temperature of 22 °C and fed diet with 1.5% SO and 18% CP; CV = coefficient of variation.
[ab] Values with different letters within a row are significantly different ($P < 0.05$).

Table 3
Performance of growing pigs kept in high-heat environments fed diets with different levels of soybean oil (SO) and crude protein (CP).

Crude protein (%)	Soybean oil (%)			Mean
	1.5	3.0	4.5	
Average daily gain (g/d)				
18.0	933	911*	966	936
15.5	888*	969	1005	954
Mean	911[b]	940[ab]	985[a]	
Control[1]	1017			
CV (%)	5.51			
Feed:gain				
18.0	2.58*	2.43	2.44	2.48
15.5	2.67*	2.57*	2.41	2.55
Mean	2.62[a]	2.50[ab]	2.42[b]	
Control[1]	2.29			
CV (%)	7.05			

[1]Control = animals kept at a temperature of 22 °C and fed diet with 1.5% SO and 18% CP; CV = coefficient of variation.
* Differ from the control ($P < 0.05$).
[ab] Values with different letters within a row are significantly different ($P < 0.05$).

was 82.2 ± 0.7 and 71.6 ± 1.1% in the high-temperature and thermoneutral environment, respectively.

3.1. Growth performance

Considering the pigs were kept under high-temperature environment, no interaction between SO and CP levels in diets was observed on average daily gain, average daily feed intake, and feed:gain (Table 2). The addition of 4.5% of soybean oil to the diet improved ADG ($P = 0.01$) and F:G ($P = 0.04$) of pigs maintained in the high-temperature environment, regardless of CP level used. The SO level did not affect the ADFI. On the other hand, the reduced CP diets (from 18.0% to 15.5%) increased ($P = 0.04$) the ADFI without affecting ADG and F:G.

Comparing pigs that received 18% CP and 1.5% SO diets with the control, it was observed that the high-temperature environment increased ($P = 0.004$) the F:G of the animals (Table 3). The same was observed when CP level was reduced to 15.5% in pigs maintained in high-temperature environment. However, when the SO levels were increased to 4.5%, the performance was similar, reducing or not the CP levels, in diets supplemented with the main amino acids.

3.2. Physiologic variables

The animals maintained in the high-temperature environment showed ($P < 0.001$) higher internal and surface body temperature and respiratory rate (Table 2). The SO addition associated with reduced CP did not influence these variables in pigs maintained in the high-temperature environment. Serum concentrations of free T3 and T4 were not affected by temperature, SO, and CP levels in the diets.

4. Discussion

The average air temperature recorded in the thermoneutral environment can be considered as thermoneutral (from 18 to 26 °C) (Muirhead and Alexander, 1997). Based on these values, it is supposed that the animals in high-temperature conditions have been under heat stress, following the objective of this work.

Despite the difference between the thermoneutral and high-temperature conditions, the air relative humidity are within the range considered normal for growing pigs (from 60% to 80%) (Whates and Whittemore, 2006). The values of BGHI were similar to rates of 83, 81 and 82 (high-temperature environment) and 69, 69 and 70 (thermoneutral environment) recorded, respectively, by Tavares et al. (2000), Orlando et al. (2001) and Kiefer et al. (2005) in studies with growing pigs.

4.1. Growth performance

The high-temperature environment reduced the animal performance with regards to the control diets.

However, the presence of higher SO levels proved to be beneficial to performance, whether or not the CP was reduced in diets containing synthetic amino acids. Spencer et al. (2005), working with growing pigs housed at temperatures between 27 and 35 °C, observed that 8% of oil in diets improved feed efficiency and ADG of animals, resulting in an 11% increase in growth rate compared to diets with 1% of oil.

It is known that diets containing higher oil levels have a lower caloric increment compared to carbohydrates, reducing the amount of heat generated in the feed digestion (Li and Sauer, 1994). The lower metabolic heat production leads to a lower energy expenditure for the homeothermy maintenance, making energy available for tissue growth. This growing of tissues explains the greater ADG observed in animals maintained in high-temperature environment fed diets with higher SO levels. In this case, the increase of SO from 1.5% to 4.5% was able to eliminate the negative effects induced by the high temperature.

In addition to the smaller caloric increment provided by higher oil diets, it is possible that the positive effect on performance may also be related to improvement in the digestibility of some amino acids. Several studies have shown that the addition of oil improves the digestibility of these nutrients, especially lysine, serine, glycine and threonine (Almeida et al., 2007; Li and Sauer, 1994). In the present work, the oil addition may have changed the amino acid ratio, which explains the similarity in performance of animals maintained in high-temperature environments fed diets with reduced CP levels (2.41 of F:G) compared to the animals maintained in thermoneutral environment (2.29 of F:G) fed diets with high oil levels. In this case, the oil inclusion may have freed non-essential amino acids, maintaining the performance of animals.

In this study, the oil levels in the diets did not affect the ADFI of animals maintained under high-temperature conditions, similar to results found by Le Bellego et al. (2002). These authors added 2% of SO in 16% CP diets for growing pigs maintained in 29 °C ambient temperature. They also observed that the reduction of CP from 20% to 16% did not affect the ADFI of animals.

In an experiment conducted by Noblet et al. (2001), it was found that reduced CP diets resulted in lower total heat production by animals, mitigating the negative effects caused by high temperatures on ADFI. In the present work, the variation of temperature was not enough to affect the feed intake by animals. However, the increase in consumption associated with a reduced CP diet may be related to the lower caloric increment of these diets.

The ambient temperature did not affect the ADFI, regardless of the levels of oil and protein used. These results differed from those presented by Tavares et al. (2000) and Manno et al. (2006), who found lower feed intake by animals maintained in high-temperature conditions. Quiniou et al. (2000) observed that the effect of ambient temperature on feed intake is quadratic, depending on the temperature range used. In the present work, the difference between ADFI showed that animals were maintained in different conditions.

With respect to F:G, Stahly and Cromwell (1979), working with growing pigs housed in three different temperatures (10, 23 and 35 °C), also observed an improvement in animals receiving isoenergetic and isonitrogenous diet with 5% of fat. This improvement was more significant under high temperature conditions. This result, as well as those obtained in the present work, may be associated with the inefficiency of pigs in dissipating corporal heat when maintained in high temperature conditions (Renaudeau et al., 2008) and also the lower caloric increment generated by the inclusion of fats in the diet (Li and Sauer, 1994).

4.2. Physiologic variables

The SO and CP levels in the diets did not affect the animals' body temperature and respiratory rate. Only the ambient temperature affected these parameters, which was expected due to the thermal stress to which the animals were subjected. Raising the temperature from 22 to 32 °C increased the surface body temperature of 6.62% of the animals, on average, by 0.64% for the internal temperature and 87% for the respiratory rate. Similar results were obtained by Manno et al. (2006) when working with animals kept in environments of 22 and 32 °C. According to Yan and Yamamoto (2000), skin temperature increase by 0.47 °C for each 1 °C of increase in ambient temperature. The authors worked with temperatures ranging from 10 to 35 °C. In the present work, where the temperature ranged from 22 to 32 °C, the increase in skin temperature was 0.28 °C on average for each 1 °C of increase in ambient temperature. This increase in skin temperature can be explained by an increased volume of blood in the vessels in order to increase heat dissipation by conduction.

Regarding the use of modified diets for animals kept in high-temperature environment, the results of this study indicate that the SO inclusion or CP reduction do not alter body temperature and respiratory rate of growing pigs.

The variation of temperature was not sufficient to alter the levels of circulating free T3 and T4. According to Becker et al. (1992), reducing the activity of the thyroid is related to the reduction of metabolic heat production in animals kept at high temperatures. In a study with piglets from 15 to 35 kg, Collin et al. (2002) observed a reduction of total T4 concentration in pigs kept in temperatures of 33 °C compared to the thermoneutral environment (23 °C). These differences in results of different studies may be related to the age of the animals and the type of hormone analysis (total or free). In the present work, the temperature range studied may not have been sufficient to alter the concentration of free T3 and T4. Moreover, according to Collin et al. (2002), there is a greater possibility of haemodilution in animals kept in thermoneutral environments compared to those under heat stress due to reduced water loss in the moments before slaughter.

The effects of thyroid hormones in thermogenesis are well known (Gregory and Berry, 1991). In the present work, a reduction of thyroid hormone concentration was expected in animals kept at high temperatures as well as the caloric increment reduction of diets formulated with the ideal protein concept (Noblet et al. 1994;

Zangeronimo et al., 2007) or with oil in substitution of carbohydrates (Baião and Lara, 2005). But in the conditions of the study, the manipulation of dietary levels of CP and SO were not sufficient to detect any significant difference in the hormone levels of animals.

Research conducted by Macari et al. (1983) with pigs in cold (10 °C) and high-temperature (35 °C) conditions observed that the energy levels in the diet influenced the serum thyroid hormones concentration, but the authors associated this effect with ADFI. The temperature had no significant effect. Others studies should be conducted with animals maintained in thermal stress conditions, mainly with reduced CP diets with amino acid inclusion, in association with high oil diets.

5. Conclusion

The increase in oil level in isocaloric diets for growing pigs improves the performance of animals kept at high temperatures. In these conditions, crude protein can be reduced, since supplemented with crystalline amino acids. Raising the temperature from 23 °C to 32 °C influences the body temperature and respiratory rate, without, however, affecting the levels of circulating free T3 and T4.

Conflict of interest statement

The research described in this manuscript has not been published before, it is not being considered for publication elsewhere, and it is free of conflict of interest.

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